



UNIVERSITI PUTRA MALAYSIA

***IN VITRO* VIABILITY AND ULTRASTRUCTURAL CHANGES
OF CRYOPRESERVED IMMATURE
BOVINE OOCYTES**

MYINT THEIN

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BOVINE OOCYTES**

By

MYINT THEIN

**Thesis Submitted to the School of Graduate Studies
Universiti Putra Malaysia in Fulfilment of the Requirements for the
Degree of Doctor of Philosophy**

January 2003

DEDICATION

**This thesis is dedicated
To My Teachers and My Parents
For their profound gratitude
And
To My Wife, Daughter and Son
For their eternal love**

Abstract of dissertation submitted to the Senate of Universiti Putra Malaysia
in fulfilment of the requirements for the degree of Doctor of Philosophy

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Chairman : Assoc. Prof. Dr. Abd. Wahid Haron, D.V.M., Ph.D.

Faculty : Veterinary Medicine

Several studies have shown that current cryopreservation procedures are severely detrimental to the viability of immature bovine oocytes and permit fertilization and development at a very reduced rate. In this study, a number of experiments were conducted to determine the *in vitro* viability of frozen-thawed and vitrified-thawed immature bovine oocytes.

In vitro viability of frozen-thawed immature bovine oocytes was determined based on cumulus mass expansion, nuclear maturation, cleavage and blastocyst rates. Viability was assessed following experiments conducted using a variety of cooling starting temperatures, seeding temperatures, permeable cryoprotectants and saccharides. Effect of using follicular fluid in the preparation of freezing solution on the viability of immature bovine oocytes was also examined. During freezing, chilling injury and

cryoprotective agents impaired the viability of immature oocytes. Among the initial cooling temperatures tested, 30°C yielded the best maturation (34.4%) and cleavage (4.5%) rates and while maturation, cleavage and blastocyst rates from unfrozen oocytes were 86.7%, 69.5% and 17.4%, respectively. As for the permeable cryoprotectants, ethylene glycol was the least toxic compared to propanediol and dimethyl sulphoxide. In the experiment of viability study of oocytes after exposure to freezing solution, significantly better cleavage and blastocyst rates were observed when follicular fluid from >15-mm follicles was added in freezing solution. However, maturation and cleavage rates following freezing with follicular fluid were statistically significant. Follicular fluid may have the beneficial effect by protecting oocytes from the toxicity of freezing solution but it may not have enough protective property against freezing *per se*.

The maturation rate of immature oocytes was severely affected when exposed to vitrification solution (39.6%) and vitrifying-thawing procedure (33.9%). However, maturation rate of vitrification solution-exposed oocytes did not differ significantly from that of vitrified-thawed oocytes. These results indicate that the adverse effect on maturation rate is mainly due to vitrification solution rather than vitrification procedure.

Any ultrastructural alterations resulted from freezing and vitrification procedures were investigated using the transmission electron microscopy in order to facilitate a better understanding of the cause of the low viability. Enlarged perivitelline space and fewer microvilli were common ultrastructural alterations that resulted from cryopreservation.

Despite impairment on the viability of oocytes, most organelles of cryopreserved oocytes were able to retain their morphology.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**KEUPAYAAN HIDUP *IN VITRO* DAN PERUBAHAN ULTRASTRUKTUR
OOSIT BOVIN TIDAK MATANG YANG DISEJUKBEKUKAN**

Oleh

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Beberapa kajian menunjukkan prosedur penyejukbekuan terkini yang dilakukan ke atas oosit bovin tidak matang mengakibatkan kegagalan keupayaan hidup yang teruk dan pengurangan kadar persenyawaan dan perkembangannya. Dalam kajian ini, beberapa ujian dilakukan untuk mengenal pasti keupayaan hidup secara *in vitro* oosit bovin tidak matang yang disejukbeku dan divitrifikasi.

Keupayaan hidup *in vitro* oosit tidak matang bovin ditentukan berdasar pengembangan jisim kumulus, pematangan nukleus, kadar pembelahan dan blastosis. Keupayaan hidup dinilai ujikaji dikendalikan secara penentuan suhu permulaan penyejukbekuan, suhu seeding, sakarid dan bahan penebat sejuk boleh serap. Kesan penggunaan cecair folikel dalam penyediaan larutan penyejukbekuan ke atas keupayaan hidup oosit tidak matang bovin juga diperiksa. Penyejukbekuan mengurangkan keupayaan hidup oosit tidak

matang. Di kalangan suhu permulaan penyejukbekuan yang diuji, 30°C memberikan kadar pematangan (34.4%) dan pembelahan (4.5%) yang terbaik sementara kadar pematangan, pembelahan dan blastosis bagi oosit yang tidak disejukbeku masing-masing adalah 86.7%, 69.5% dan 17.4%. Bagi larutan penyejukbekuan mudah resap, etilene glikol didapati sangat kurang toksik berbanding propanediol dan dimetil sulfoksida. Dalam ujian keupayaan hidup oosit selepas terdedah kepada larutan pembekuan, kadar pembelahan dan blastosis yang lebih bererti diperolehi apabila cecair folikel bersaiz >15mm dicampurkan dalam larutan pembekuan. Walau bagaimanapun, tiada perbezaan dalam kadar pematangan dan pembelahan diperolehi selepas disejukbeku dengan cecair folikel. Cecair folikel berkemungkinan mempunyai kesan baik untuk melindungi oosit daripada kesan toksik larutan pembekuan tetapi tidak mengandungi keupayaan pelindung terhadap pembekuan.

Kadar pematangan oosit yang tidak matang sangat terjejas dengan pendedahan larutan vitrifikasi (39.6%) dan prosedur nyahvitrifikasi (33.9%). Kadar pematangan di antara oosit terdedah larutan vitrifikasi dan vitrifikasi tidak menunjukkan perbezaan. Keputusan ini menunjukkan kesan terjejas terhadap pematangan adalah berpunca dari larutan vitrifikasi dan bukannya prosedur vitrifikasi.

Perubahan ultrastruktur berpunca dari pembekuan dan vitrifikasi disiasat menggunakan mikroskop elektron transmisi dalam usaha untuk memperoleh jawapan dan penerangan terhadap keupayaan hidup yang rendah. Ruang perivitellin yang besar dan sedikit mikrovilli adalah perubahan ultrastruktur yang lazim berpunca dari penyejukbekuan.

Sungguhpun kemampuan hidup amat terjejas, kebanyakan organel oosit yang disejukkan berupaya mengekalkan morfologi mereka.

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I certify that an Examination Committee met on 27th January 2003 to conduct the final examination of Myint Thein on his Doctor of Philosophy thesis entitled “*In Vitro* Viability and Ultrastructural Changes of Cryopreserved Immature Bovine Oocytes” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



Myint Thein

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TABLE OF CONTENTS

	Page
DEDICATION.....	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL SHEETS.....	xi
DECLARATION FORM	xiii
LIST OF TABLES	xvii
LIST OF FIGURES.....	xix
LIST OF ABBREVIATIONS	xxiv

CHAPTER

I	GENERAL INTRODUCTION	1
II	LITERATURE REVIEW	5
	2.1 Introduction	5
	2.2 <i>In Vitro</i> Production of Embryos.....	6
	2.2.1 <i>In Vitro</i> Maturation.....	7
	2.2.2 <i>In Vitro</i> Fertilization.....	8
	2.2.3 <i>In Vitro</i> Culture.....	10
	2.2.4 Embryo Transfer and Embryo Freezing.....	11
	2.3 <i>In Vitro</i> Viability Assessment of Oocytes.....	12
	2.4 Cryopreservation.....	16
	2.4.1 Cryopreserved Alive.....	16
	2.4.2 Oocyte Cryopreservation.....	19
	2.4.3 Cryoprotectants.....	23
	2.4.4 Principles of Cryopreservation.....	33
	2.5 Freezing.....	34
	2.5.1 Definition.....	34
	2.5.2 Ice Crystal Formation.....	35
	2.5.3 Seeding.....	36
	2.5.4 Freezing Injuries.....	38
	2.5.5 Thawing.....	40
	2.6 Vitrification.....	42
	2.6.1 Definition.....	42
	2.6.2 Vitrification versus Equilibrium Freezing.....	43
	2.6.3 Recent Development.....	46

2.6.4	Future Prospects.....	48
2.7	Ultrastructure of Immature Bovine Oocytes.....	49
2.7.1	Cumulus and Corona Radiata Cells.....	50
2.7.2	Zona Pellucida.....	51
2.7.3	Ooplasm.....	52
III	GENERAL MATERIALS AND METHODS.....	55
3.1	Samples and Resources.....	55
3.2	Ancillary Procedures.....	56
3.2.1	Washing and Cleaning.....	56
3.2.2	Sterilization.....	56
3.3	<i>In Vitro</i> Production of Embryos.....	57
3.3.1	Recovery of Oocytes.....	57
3.3.2	<i>In Vitro</i> Maturation.....	58
3.3.3	<i>In Vitro</i> Fertilization.....	59
3.3.4	<i>In Vitro</i> Culture.....	60
3.3.5	<i>In Vitro</i> Viability Assessment.....	62
3.4	Cryopreservation of Immature Bovine Oocytes.....	65
3.4.1	Freezing and Thawing.....	65
3.4.2	Vitrification.....	66
3.5	Transmission Electron Microscopy.....	69
3.5.1	Fixation.....	69
3.5.2	Postfixation.....	70
3.5.3	Dehydration.....	70
3.5.4	Infiltration and Embedding.....	71
3.5.5	Sectioning and Preparation of Grids.....	72
3.5.6	Double Staining with Uranyl Acetate and Lead Citrate.....	74
3.6	Statistical Analyses.....	75
IV	<i>IN VITRO</i> VIABILITY OF FROZEN-THAWED IMMATURE BOVINE OOCYTES.....	76
4.1	Introduction.....	76
4.2	Experiments.....	78
4.2.1	Effect of Initial Cooling Temperature on Viability of Immature Bovine Oocytes.....	79
4.2.2	Effect of Seeding Temperature on Developmental Competence of Frozen-thawed Bovine Oocytes.....	97
4.2.3	Effect of Cryoprotectants on Viability of Immature Bovine Oocytes.....	100
4.2.4	Effect of Freezing Solution Exposure and Freezing-thawing Procedure.....	111
4.2.5	Effect of Follicular Fluid Supplementation in Freezing Solution on Viability of Bovine Oocytes	121

V	<i>IN VITRO</i> VIABILITY OF VITRIFIED-THAWED IMMATURE BOVINE OOCYTES.....	133
	5.1 Introduction.....	133
	5.2 Experiments.....	134
	5.2.1 <i>In Vitro</i> Maturation of Bovine Oocytes Following Exposure to Vitrification Solution and Vitrifying-thawing Procedure....	134
	5.2.2 Open Pulled Straw (OPS) and Glass Micropipette (GMP) Vitrification.....	139
	5.2.3 Effect of Follicular Fluid Supplementation in Vitrification Solution on Viability of Bovine Oocytes.....	145
VI	MICROSCOPIC AND ULTRASTRUCTURAL ALTERATIONS OF CRYOPRESERVED-THAWED IMMATURE BOVINE OOCYTES.....	152
	6.1 Introduction.....	152
	6.2 Experiments.....	153
	6.2.1 Morphological Study of Cryopreserved Immature Bovine Oocytes Under Light Microscope.....	154
	6.2.2 Ultrastuctural Study of Cryopreserved Immature Bovine Oocytes Under Transmission Electron Microscope.....	159
VII	GENERAL DISCUSSION.....	171
VIII	SUMMARY AND CONCLUSIONS.....	176
	REFERENCES.....	180
	APPENDICES	
	Appendix A.....	202
	Appendix B.....	209
	Appendix C.....	211
	Appendix D.....	216
	Appendix E.....	219
	BIODATA.....	223

LIST OF TABLES

Table	Page
4.1 Cumulus expansion and maturation rates of frozen-thawed immature bovine oocytes with different cooling starting temperature.....	84
4.2 Developmental capacity of fresh and frozen-thawed immature bovine oocytes.....	85
4.3 Developmental capacity of frozen-thawed immature bovine oocytes derived from various seeding temperatures in EG.....	99
4.4 Developmental capacity of control and freezing solution-exposed oocytes (intracellular cryoprotectants).....	104
4.5 Cumulus expansion rate and developmental capacity of freezing solution-exposed oocytes (sugars).....	105
4.6 Cumulus expansion and maturation rates of freezing solution-exposed and frozen-thawed immature bovine oocytes.....	114
4.7 Developmental capacity of freezing solution-exposed and frozen-thawed immature bovine oocytes.....	115
4.8 Developmental competence of immature bovine oocytes following exposure to freezing solution supplemented with follicular fluid.....	124
4.9 Cumulus expansion and maturation rates of frozen-thawed immature bovine oocytes of EG group and EG+FF group.....	125
4.10 Developmental competence of frozen-thawed immature bovine oocytes (using freezing solution supplemented with follicular fluid)...	126
4.11 Summary table for Chapter IV.....	131
5.1 Cumulus expansion and maturation rates of immature bovine oocytes following vitrification solution exposure and vitrifying-thawing procedure.....	136
5.2 Developmental capacity of OPS and GMP vitrified-thawed immature bovine oocytes.....	141

5.3	Cumulus expansion and maturation rates of immature bovine oocytes following exposure to vitrification solution formulated with follicular fluid.....	147
5.4	Developmental capacity of vitrified-thawed immature bovine oocytes after using follicular fluid.....	149
5.5	Summary table for Chapter V.....	151
6.1	Changes in microscopic appearance of cryopreserved-thawed immature bovine oocytes.....	157
6.2	Numbers (%) of oocytes exhibiting ultrastructural abnormalities following freezing and vitrification.....	164
6.3	Summary table for Chapter VI.....	170

LIST OF FIGURES

Figure	Page
4.1 Morphological appearance of a good quality cumulus-oocyte-complex (COC), X200.....	86
4.2 A discarded oocyte. Note the heterogeneous appearance of ooplasm (arrow), X200.....	86
4.3 A fibrinated oocyte (discarded oocyte). Note the expansion of cumulus cells (arrow), X200.....	87
4.4 Partially denuded oocyte. Note coarse granules in heterogeneous ooplasm, misshapen outline of oolema(arrow), X200.....	87
4.5 A discarded COC (dark ooplasm indicating degeneration and vacuolations indicating damage to the cytoskeleton), X200.....	88
4.6 A denuded oocyte. Note frayed marginated zona (arrow head), absence of intact cytoplasmic membrane (thin arrow) and incompact ooplasm, X200.....	88
4.7 Full cumulus expansion (cumulus mass expanded to at least 3 folds of an oocyte diameter), X200.....	89
4.8 Moderate cumulus expansion (cumulus mass expanded to approximately 2 folds of an oocyte diameter), X200.....	89
4.9 Slight cumulus expansion (cumulus expanded to less than one oocyte diameter), X200.....	90
4.10 No cumulus expansion (cumulus mass remained tight and adherent to the periphery of an oocyte), X200.....	90
4.11 <i>In vitro</i> matured oocyte derived from frozen-thawed group (note the shining zone around the M II plate is larger than that of a normal one, X400.....	91
4.12 Bovine cumulus-oocyte-complexes following recovery, X40.....	91
4.13 Frozen-thawed oocytes from initial cooling temperature 30°C group, X40.....	92

4.14	Frozen-thawed oocytes from initial cooling temperature -6°C group, X40.....	92
4.15	Oocytes from frozen-thawed group after removal of cumulus cells at 18 hours post insemination (note enlarged perivitelline space, empty zonae, broken cytoplasmic membrane and transparent cytoplasm), X40.....	93
4.16	Several spermatozoa (arrows) inside the enlarged perivitelline space of a frozen-thawed oocyte. X320.....	93
4.17	Cleaved bovine embryos derived from fresh (control) oocytes at 48 hours post insemination (note the spermatozoa around the zona), X200...	94
4.18	Cleaved embryos and degenerated oocytes derived from frozen-thawed immature bovine oocytes. Note the transparent ooplasm (arrow) of uncleaved frozen-thawed oocytes), X200.....	94
4.19	Blastocysts derived from bovine oocytes of control group, X40.....	95
4.20	Morphological appearance of an expanded bovine blastocyst. Note well-defined blastocoele (thin arrow) and darker inner cell mass (block arrow), X320.....	95
4.21	Presumptive zygotes derived from freezing solution-exposed oocytes (just after removal of cumulus cells at 18 hours post insemination), X40.	105
4.22	Cleaved early embryos derived from DMSO-exposed oocytes (just after transfer from temporary medium into BOEC culture medium at 48 hours post insemination), X40.....	106
4.23	A presumptive zygote with first polar body (arrow) from DMSO-exposed group at 20 hours post insemination (second polar body has not been extruded yet), X320.....	106
4.24	A presumptive with first and second polar bodies at 20 hours post insemination, X320.....	107
4.25	A presumptive zygote from freezing solution-exposed group at 20 hours post insemination (note disintegrated polar body/bodies, extruded cytoplasmic bodies, incompact ooplasm and shrunk oolema), X320.....	107
4.26	Cleaved embryos derived from EG-exposed oocytes at day 3 post insemination (note a misshapen zona outline of an embryo with a good kinetic cell division). X200.....	108
4.27	An early morula at day 5 post-insemination (derived from EG-exposed group), X320.....	108

4.28	An expanded blastocyst and degenerated embryos derived from EG-exposed oocytes (note the BOEC monolayer as background and an adjacent misshapen embryo), X200.....	109
4.29	An oocyte with first anaphase spindle (arrow) derived from freezing solution-exposed group at 24 h <i>in vitro</i> maturation, X400.....	116
4.30	Second metaphase plate (thick arrow) and polar body (thin arrow) of an <i>in vitro</i> matured oocyte derived from freezing solution-exposed group, X400.....	116
4.31	Metaphase II chromosome plate and polar body of an <i>in vitro</i> matured oocyte derived from frozen-thawed group at 24 h IVM (note incompact polar body), X400.....	117
4.32	Further division of the metaphase II spindles and formation of two identical anaphase II spindles (arrows) at 24 h IVM (derived from a frozen-thawed oocyte), X400.....	117
4.33	An expanded blastocyst derived from fresh oocytes (note the typical features: 1.2-1.5X increase in diameter, thinning of zona to 1/3 of its original thickness, well defined blastocoele, dark and compact cell mass), X200.....	126
4.34	Expanded and hatched blastocysts derived from freezing solution (EG+FF)-exposed oocytes at day 8 post-insemination (note the BOEC monolayer as background), X40.....	127
4.35	Hatching blastocyst at day 8 post-insemination, observed from freezing solution (EG+FF)-exposed group (note adjacent early blastocyst and degenerated embryos), X200.....	127
4.36	A hatched blastocyst derived from freezing (EG+FF)-exposed group (note typical inner cell mass (arroe) and trophoblast but relatively large extruded cell mass left in zona (arrow head), X200.....	128
5.1	Second metaphase plate observed in an IVM oocyte following exposure to vitrification solution (note relatively big shining zone around the chromosomes), X400.....	136
5.2	Dispersed MII chromosomes (D) of an <i>in vitro</i> matured oocyte following exposure to vitrification solution, X400.....	137
5.3	Improper spindle of an oocyte at 24 h IVM following exposure to vitrification solution, X400.....	137

5.4	An OPS vitrified-thawed oocyte. Note undamaged cumulus attachment (Cm), normal appearance of zona pellucida (Zp) and homogenous ooplasm, X200.....	142
5.5	A GMP vitrified-thawed oocyte. Note the heterogenous appearance of ooplasm (arrow), X200.....	142
5.6	A misshapen cleaved embryo derived from the GMP vitrified-thawed immature bovine oocyte (observed at 72 hours post insemination, note the irregular outline of zona pellucida), X320.....	143
5.7	An IVM oocyte derived from VSF-exposed immature bovine COC (note disorganized chromosomes surrounding by a well demarcated shining zone), X400.....	148
6.1	A VS-exposed cumulus-oocyte-complex. The enlarge perivitelline space and less numbers of microvilli (block arrow) are apparent. The zona pellucida (ZP) is traversed by cytoplasmic processes (thin arrows). Most corona radiata and cumulus cells (CC) retain their normal ultrastructure and seldom contain cytoplasmic vacuoles. X3150.....	164
6.2	A control oocyte with intact germinal vesicle (GV), relatively uniformed vesicles (V), peripherally located mitochondria (m) and zona pellucida (ZP). X2000.....	165
6.3	A VS-exposed oocyte showing normal zona pellucida, normal cluster of cortical granules (arrows) and abnormally big vesicle (V). X3150.....	165
6.4	A group of cortical granules (CG) with electron densities including a vacuolated cortical granule (arrow), moderately enlarged perivitelline space (PV) and several microvilli (mv). X2500.....	166
6.5	An enlarged lipid droplet from a frozen-thawed immature bovine oocyte. Zona pellucida (ZP), perivitelline space (pv) and vesicles (V) are shown for the reference. X6300.....	166
6.6	Swollen Golgi complexes (GC) and normal mitochondria. X50,000.....	167
A.1	Ovary collection at Senawang abattoir.....	219
A.2	Recovery of immature bovine oocytes by aspiration.....	219
A.3	Oocytes searching under a stereomicroscope.....	220
A.4	Programmable freezer (Freeze control® CL-683, Cryologic, Pty Ltd, Australia).....	220

A.5	Programmable freezer (Freeze control and cryo bath assembled).....	221
A.6	Liquid nitrogen tank for storing frozen oocytes.....	221
A.7	Vortexing cumulus-oocyte-complexes (COCs) to strip off cumulus cells.....	222
A.8	CO ₂ incubator (HERA cell, Kendro Laboratory Products GmbH, Germany).....	222

LIST OF ABBREVIATIONS

AFP	Antifreeze protein
A I	Anaphase I (First anaphase)
ART	Assisted reproductive technology(ies)
ATP	Adenosine triphosphate
BG	1,3-butylene glycol
BOEC	Bovine oviduct epithelial cell
BSA	Bovine serum albumin
CG	Cortical granule
CL	Corpus luteum
COCs	Cumulus-oocyte-complexes
conc	Concentration
CPA	Cryoprotective Agent
CS	Calf serum
DEG	Diethylene glycol
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
D-PBS	Dulbecco's phosphate-buffered saline
dpi	Day(s) post-insemination
EG	Ethylene Glycol
EGF	Epidermal growth factor
EME	Ethylene glycol monomethyl ether